

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Original) A method for introducing into a subject a population of stem cells having partial or complete loss of function of a target gene, the method comprising:
  - a) introducing a nucleic acid construct encoding an shRNA into stem cells to generate transfected stem cells, wherein the shRNA is complementary to a portion of the target gene;
  - b) introducing the transfected stem cells into the subject,wherein the transfected stem cells propagate within the subject and retain partial to complete loss of function of the target gene.
2. (Original) The method of claim 1, wherein the target gene participates in a disease process in the subject.
3. (Original) The method of claim 2, wherein the target gene encodes a host protein that is co-opted by a virus during viral infection.
4. (Original) The method of claim 3, wherein the host protein is a cell surface receptor for a virus.
5. (Original) The method of claim 4, wherein the virus is a human immunodeficiency virus.
6. (Original) The method of claim 2, wherein the target gene is a gene encoding a polypeptide of a Major Histocompatibility Complex.
7. (Original) The method of claim 1, wherein the transfected cells replace a population of diseased cells in the subject.
8. (Original) The method of claim 1, wherein the transfected cells are autologous cells derived from cells of the subject.
9. (Original) The method of claim 1, wherein the subject is a human patient.
10. (Original) The method of claim 1, wherein the shRNA is expressed constitutively.
11. (Original) The method of claim 1, wherein shRNA expression is conditional.

12. (Original) The method of claim 11, wherein expression of the shRNA is conditional on the presence or absence of a substance administered to the subject.
13. (Original) The method of claim 1, wherein the shRNA expression is cell lineage specific.
14. (Original) The method of claim 1, wherein the stem cells are hematopoietic stem cells.
15. (Original) The method of claim 14, wherein endogenous hematopoietic stem cells of the subject are ablated.
16. (Original) The method of claim 1, wherein the stem cells are embryonic stem cells.
17. (Original) The method of claim 1, wherein the transfected stem cells are cultured so as to generate a population of further differentiated transfected stem cells for introduction into the subject.
18. (Original) The method of claim 1, wherein the subject is a mouse.
19. (Original) The method of claim 1, wherein the nucleic acid construct is a retroviral vector.
20. (Original) The method of claim 18, wherein the nucleic acid construct is a lentiviral construct.
21. (Original) The method of claim 1, wherein the nucleic acid construct is derived from a Murine Stem Cell Virus (MSCV).
22. (Original) The method of claim 1, wherein the vector is a human ex vivo gene therapy vector.
23. (Original) The method of claim 1, further comprising verifying the partial or complete loss of function of the target gene prior to introducing the transfected cells into the subject.
24. (Original) A method for introducing into a subject a population of differentiated cells having partial or complete loss of function of a target gene, the method comprising:
  - a) introducing a nucleic acid construct encoding an shRNA into stem cells to generate transfected stem cells, wherein the shRNA is complementary to a portion of the target gene;
  - b) culturing the transfected stem cells to generate transfected differentiated cells having partial or complete loss of function of a target gene; and

- c) introducing the transfected differentiated cells into the subject,  
wherein the transfected differentiated cells retain partial to complete loss of function of the target gene.
25. (Original) A method of treating a disease associated with the expression of a target gene in a population of cells, the method comprising:
- a) introducing a nucleic acid construct encoding an shRNA into stem cells to generate transfected stem cells, wherein the shRNA is complementary to a portion of the target gene;
- b) introducing the transfected stem cells into the subject,  
wherein the transfected stem cells propagate within the subject and retain partial to complete loss of function of the target gene.
26. (Original) The method of claim 25, wherein the target gene has cell autonomous effects that contribute to the disease.
27. (Original) The method of claim 25, wherein the population of cells, or progenitor cells thereof, are ablated prior to introducing the stem cells into the subject.
28. (Original) The method of claim 25, wherein the stem cells are hematopoietic stem cells.
29. (Currently Amended) The method of claim ~~29~~ 25, wherein the disease is a dominant genetic disease.
30. (Original) The method of claim 29, wherein the dominant genetic disease is caused by a gain of function mutation.
31. (Original) A non-human mammal comprising a population of stem cells comprising a nucleic acid construct encoding an shRNA, or progeny cells thereof, wherein the cells exhibit partial to complete loss of function of a target gene.
32. (Original) The non-human mammal of claim 31, wherein the non-human mammal is a mouse.
33. (Original) A composition formulated for administration to a human patient, the composition comprising:

a) a stem cell comprising a nucleic acid construct encoding an shRNA, wherein the shRNA is complementary to at least a portion of a target gene, and wherein the cells exhibit partial to complete loss of function of a target gene; and

b) a pharmaceutically acceptable excipient.

34. (Original) The composition of claim 33, wherein the stem cell is a hematopoietic stem cell.

35. (Original) A method for identifying a gene that affects the sensitivity of tumor cells to a chemotherapeutic agent, the method comprising:

a) introducing into a subject a transfected stem cell comprising a nucleic acid construct encoding an shRNA, wherein the shRNA is complementary to at least a portion of a target gene, wherein the transfected stem cell exhibits decreased expression of the target gene, and wherein the transfected stem cell gives rise to a transfected tumor cell in vivo;

b) evaluating the effect of the chemotherapeutic agent on the transfected tumor cell.

36. (Original) The method of claim 35, wherein evaluating the effect of the chemotherapeutic agent on the transfected tumor cell comprises: administering the chemotherapeutic agent to the subject and measuring the quantity of tumor cells derived from the transfected stem cell.

37. (Original) The method of claim 36, further comprising comparing the quantity of tumor cells derived from the transfected stem cell to the quantity of tumor cells derived from the transfected stem cell in a control subject that has not received the chemotherapeutic agent.

38. (Original) A method for identifying a gene that affects the sensitivity of tumor cells to a chemotherapeutic agent, the method comprising:

a) introducing into a subject a plurality of transfected stem cells, wherein each transfected stem cell comprises a nucleic acid construct comprising a representative shRNA of an shRNA library, and wherein a representative shRNA of an shRNA library is complementary to at least a portion of a representative target gene, wherein a plurality of the transfected stem cells exhibits decreased expression of a representative target gene, and wherein a plurality of the transfected stem cells gives rise to transfected tumor cells in vivo;

b) administering a chemotherapeutic agent; and

c) identifying representative shRNAs that are enriched or depleted by treatment with the therapeutic agent.

39. (Original) The method of claim 38, wherein a representative shRNA is associated with a distinguishable tag.

40. (Original) The method of claim 39, wherein the shRNA library is a barcoded shRNA library.

41. (Original) A method of administering a chemotherapeutic agent to a patient, the method comprising:

a) administering the chemotherapeutic agent; and

b) administering a nucleic acid that causes RNA interference of a gene that is associated with chemotherapeutic resistance.

42. (Original) The method of claim 41, wherein the gene that is associated with chemotherapeutic resistance is selected from among: Bim and Puma.

43. (Original) A barcoded shRNA library comprising a plurality of representative shRNAs, wherein the majority of representative shRNAs are associated with a barcode tag.

44. (Original) The barcoded shRNA library, wherein the representative shRNAs are partially complementary to representative genes, and wherein a majority of representative gene are known or suspected to be involved in a cancer.

45. (Original) A method of determining a function of a gene comprising:

a) introducing small hairpin RNA which targets mRNA of the gene into cells;

b) maintaining the cells under conditions in which the small hairpin RNA is stably expressed and RNA interference of the mRNA occurs;

c) introducing the cells into a non-human mammal, thereby producing a knockout non-human mammal; and

d) assessing the phenotype of the knock-out non-human mammal compared to a control mammal,

thereby identifying a function of the gene.

46. (Original) The method of Claim 45 wherein the non-human mammal is a mouse.
47. (Original) A method of determining the contribution of a gene to a condition comprising:
- a) introducing small hairpin RNA which vary in their ability to inactivate mRNA of the gene into cells, thereby producing a panel of a discrete set of cells in which the mRNA of the gene is inactivated to varying degrees in each set of cells;
  - b) maintaining the cells under conditions in which the small hairpin RNA is stably expressed and RNA interference of the mRNA occurs;
  - c) introducing each set of cells into a separate non-human mammal, thereby producing a panel of knockout non-human mammals in which the mRNA of the gene is inactivated to varying degrees in each non-human mammal; and
  - d) assessing the phenotype of each knock-out non-human mammal compared to a control mammal,
- thereby determining the contribution of the gene to the condition.
48. (Original) The method of Claim 47 wherein the gene encodes p53.
49. (Original) The method of Claim 14 wherein the non-human mammal is a mouse.
50. (Original) A method of engineering cells ex vivo so that the cells exhibit reduced expression of a gene product comprising:
- a) removing cells from a host; and
  - b) introducing a construct encoding a small hairpin RNA into the cells such that the small RNA is stably expressed and induces RNA interference of the gene product.
51. (Original) The method of claim 50 wherein the gene product is of therapeutic relevance.
52. (Original) A method of claim 50 wherein the engineered cells are introduced into a human.
53. (Original) A method of claim 50 wherein the cells are derived from an individual to whom the cells are administered.
54. (Original) A method of claim 50 wherein the cells are derived from a heterologous donor.

55. (Original) A method of claim 50 wherein the heterologous donor is a different species than the species who receives the cells.

56. (Original) A method for introducing into a subject a population of stem cells having partial or complete loss of function of a target gene, the method comprising:

a) introducing a nucleic acid construct encoding an shRNA into stem cells to generate transfected stem cells, wherein the shRNA is complementary to a portion of the target gene, such that expression of the target gene is decreased;

b) removing or inactivating the nucleic acid construct;

c) verifying that expression of the target gene remains decreased;

d) introducing the stem cells into a subject,

wherein the stem cells propagate within the subject and retain partial to complete loss of function of the target gene.

57. (Original) The method of claim 56, wherein the nucleic acid construct comprises a lox site and wherein removing or inactivating the nucleic acid construct comprises introducing or activating Cre.